



## Complementary (secondary) metabolites in an octocoral competing with a scleractinian coral: effects of varying nutrient regimes

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### Abstract

Competitive interactions between two sessile, epibenthic species were investigated on the Great Barrier Reef (GBR) in the presence and absence of added nutrients, as part of the Enrichment of Nutrients on Coral Reefs Experiment (ENCORE). *Sarcophyton ehrenbergi* Marenzeller (Octocorallia: Alcyonacea), an alcyonacean soft coral, and *Pocillopora damicornis* (Linnaeus), a scleractinian coral, were relocated and placed in contact with each other on large plastic grids on each of 12 micro-atolls within the One Tree Island (OTI) lagoon (23°30'S, 152°96'E, GBR). These micro-atolls were allocated in equal-sized groups to three enrichment treatments (addition of nitrogen, N; addition of phosphorus, P; addition of both nitrogen and phosphorus, N+P) and one control. Non-relocated (NR) and relocated colonies were also monitored as controls. After relocation and 1 year of nutrient enrichment, concentrations of a terpenoid complementary metabolite—sarcophytoxide—and wax esters were analyzed in colonies of *S. ehrenbergi* that had been exposed to elevated concentrations of N, P, N+P and compared with colonies on the non-nutrient-enriched control. Non-relocated control colonies from the natural environment were monitored over a period of 1 year and compared to colonies relocated to the control micro-atolls to assess handling effects. Analyses were performed on non-interacting *S. ehrenbergi* colonies, *S. ehrenbergi* colonies in experimental contact with *P. damicornis* colonies, and on non-interacting *S. ehrenbergi* colonies from the site of initial collection. Significant differences were found between sarcophytoxide levels in colonies of *S. ehrenbergi* in contact with *P. damicornis* vs. control/non-contact colonies; contact colonies had higher levels of this

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metabolite. Non-relocated control colonies of *S. ehrenbergi* exhibited significantly higher levels of sarcophytoxide than relocated control colonies. Augmentation of nutrient levels on the micro-atolls significantly increased sarcophytoxide levels in *S. ehrenbergi* colonies relative to colonies on the control micro-atolls, although this response was not strong. Concentrations of fatty esters increased significantly through time in *S. ehrenbergi* colonies in their natural setting (non-relocated controls). This variability was not observed in relocated colonies in the treatment and control micro-atolls, irrespective of contact with *P. damicornis*. Concentrations of fatty esters in colonies of *S. ehrenbergi* in contact with *P. damicornis* were significantly lower than control/non-contact colonies, indicating that there is a cost in terms of stored energy reserves for the production of additional complementary metabolites when involved in competition for space. Augmentation of P levels in micro-atolls induced significant increases in fatty ester levels within *S. ehrenbergi* colonies vs. colonies in control micro-atolls, or in micro-atolls treated with added N or N+P together. These findings indicate that interspecific competition for space between a scleractinian coral and an alcyonacean soft coral and/or changes in the environmental nutrient regime can influence concentrations of complementary/secondary metabolites in the alcyonacean coral and the organism's stored energy reserves.

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## 1. Introduction

Soft corals are one of the most important groups of invertebrates on the Great Barrier Reef (GBR; Dinesen, 1983). They produce an array of natural products, sometimes called complementary (secondary) metabolites (Sammarco and Coll, 1997), which can play important roles in the complex behavioral and ecological interactions among organisms (Fishelson, 1970; Whittaker and Feeny, 1971; Tursch et al., 1978; Bakus, 1981; Bakus et al., 1986; Coll and Sammarco, 1983; Sammarco and Coll, 1988). The high concentrations of diterpenes found in the tissues of soft corals (Tursch et al., 1978; Coll, 1992; Faulkner, 2000) have been shown to play important roles in strategies used by soft corals against organisms competing for space on a coral reef (La Barre et al., 1986; Sammarco and Coll, 1988; Van Alstyne and Paul, 1988; Van Alstyne et al., 1994; Slattery and McClintock, 1995; Slattery et al., 1999).

Many studies have been carried out on the allelopathic effects observed in interactions between alcyonacean and scleractinian corals (La Barre and Coll, 1982; Sammarco et al., 1983, 1985; Coll and Sammarco, 1983, 1992; De Ruyter van Stevenick et al., 1988; Coll, 1992; Sammarco and Coll, 1990). These effects include retardation of growth and tissue necrosis or mortality of scleractinians via direct tissue-to-tissue contact. Mortality may also occur as a result of the passage of allelochemicals (Whittaker and Feeny, 1971) through the water column in the absence of contact (Coll et al., 1982; Schulte et al., 1991). In this way, soft corals are able to maintain and expand their living space (Sammarco et al., 1983).

The competitive balance between certain species of corals can also be controlled by environmental factors (i.e., temperature, wave action, light, predation, food, etc.; Alino et al., 1992; Coll, 1992). Indeed, the higher nutrient concentrations in inshore waters of the GBR (Sammarco and Crenshaw, 1984; Risk et al., 1994; Sammarco et al., 1999; Devlin et

al., 2000; Lourey et al., 2001; and others) may contribute to the dominance and possible expansion by octocorals in this region (Alino et al., 1992; Fabricius and Dommissie, 2000; also see Ben-David-Zaslow et al., 1999).

Variation in concentrations of sarcophytoxide, a compound known to be toxic (Tursch et al., 1978; Bowden et al., 1987), between different colonies of *Sarcophyton ehrenbergi* at One Tree Island (OTI), Great Barrier Reef, Australia, encouraged us to investigate whether we might observe any effects of nutrients on the concentration of several complementary metabolites of *S. ehrenbergi* involved in competitive interactions with the hard coral *Pocillopora damicornis*. As well as the cembranoid diterpene sarcophytoxide, wax esters (of which cetyl palmitate is a common constituent) were quantitatively analysed. This was done to provide an indicator of variation in energy storage compounds. There has been no comparable long-term in situ experiment, which has investigated whether soft corals modify their chemical composition and their ability to exert allelopathic effects on neighbouring organisms under the influence of elevated concentrations of nutrients.

## 2. Material and methods

### 2.1. Study site and experimental design

These experiments were part of a large-scale, wide-spectrum field experiment entitled “the Enrichment of Nutrients on Coral Reefs Experiment” (ENCORE; see Steven and Larkum, 1993; Larkum and Steven, 1994; McGill and Steven, 1994). Our experiment was performed on 12 micro-atolls within the One Tree Island lagoon at the southern end of the GBR (23°30'S, 152°96'E), during 1 annual cycle (March 1995, July 1995, September 1995 and February 1996). The experiment was broken into two parts. The first was a four-way, nested Model-I orthogonal design. The first factor was experimental variation in nutrient concentrations. The nutrients used were nitrogen, as ammonium chloride (NH<sub>4</sub>Cl), and phosphorus, as potassium di-hydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>). The sub-treatments within this factor were 20 μM N added to a set of micro-atolls (see below), 4 μM P added to another set of micro-atolls and a combination of these amounts (N+P) added to a third set of micro-atolls. A fourth set of micro-atolls received no nutrient additions, as a control. Nutrient treatments involved additions twice daily, commencing on each low tide, with two further additions 1 and 2 h after low water. Details of the ENCORE protocol may be found in the above references.

Nested replication represented the second factor, with each of the above treatments being replicated via three independent micro-atolls.

The third factor was a set of colonies of *S. ehrenbergi*, which had been transplanted from a separate locale onto each of the above 12 micro-atolls. Three colonies were relocated (transplanted), as is, to each micro-atoll. An additional three colonies were transplanted into contact with colonies of *P. damicornis*, to examine the response of the soft coral to competition for space under the influence of these varying nutrient regimes.

The fourth factor was time. Samples were collected in March, July and September of 1995, and February 1996.

A second sub-experiment was conducted to control for the effects of relocation of soft coral colonies. This followed a two-way Model I orthogonal design. Here, six colonies (NR) from the original collection site were monitored. Samples were taken over the same general period, using three sampling times—July and September 1995, and February 1996. They were compared with the relocated, non-contact controls.

## 2.2. Sample collection

*S. ehrenbergi* colonies attached to hard substrate were collected from the entrance of the lagoon, attached to heavy plastic grids and labeled as two sets of three colonies in each of the ENCORE micro-atolls (see Tentori et al., 1997; Fleury et al., 2000). *P. damicornis* (Hexacorallia, Scleractinia) colonies, obtained from the outer wall of the micro-atolls, were placed in contact with *S. ehrenbergi* on one of the two grids in each micro-atoll. In order to reduce the stress associated with transplanting the organisms from one environment to another, soft coral colonies were first transplanted temporarily to a separate micro-atoll for 3 months prior to placement on the experimental micro-atolls. This experiment began in March 1995, under the nutrient regime commenced in September 1994.

From the six *S. ehrenbergi* colonies transplanted to each micro-atoll, and from the six NR colonies tagged at the entrance to the lagoon, ~ 2-cm-thick cake-like slices were cut from the edges into the disc, and to the base of the stem, and removed. This was done on four occasions: March 1995, July 1995, September 1995 and February 1996. Repeated sampling from the same colony has been shown to have no effect on metabolite concentrations through time (Leone, 1993). NR samples were only collected on the last three occasions.

## 2.3. Chemical and statistical analyses

The crude extracts from two paired samples per micro-atoll (i.e., samples from three isolated *S. ehrenbergi* [S] colonies and from three *S. ehrenbergi* in contact with *P. damicornis* [SP] colonies) and from six NR colonies, were prepared for quantitative analysis by <sup>1</sup>H-NMR spectroscopy. Identification of the diterpene sarcophytoxide and of wax esters, including cetyl palmitate, in the crude extracts was confirmed by mass spectrometry coupled with gas chromatography. Details of isolation and identification of the complementary metabolite, sarcophytoxide (Bowden et al., 1987), analysed in this work and of wax esters such as cetyl palmitate (Vanderah et al., 1978), including the quantitative methodology applied, have been described previously (Leone et al., 1995; Fleury et al., 2000).

This experiment produced a large number of samples (312). Because of the cost-and labor-intensiveness of sample processing via H-NMR, it was necessary in many cases to pool replicates within a sub-treatment. This reduced the overall sample size to 104. The chemical concentrations from these pooled samples then afforded an average and the sample size was concomitantly lowered. This, of course, reduces the Power of the Test (Sokal and Rohlf, 1981), making the statistical analyses more conservative than if all samples had been kept separate. Thus, the results must be more robust in order to yield a significant result.

All percent-concentration data were transformed by arcsine for purposes of normalization prior to analysis and graphing (Zar, 1984; Sokal and Rohlf, 1981). In some cases where sample size were unequal due to missing samples, some replicates were omitted from

statistical analyses to allow analysis by multi-way ANOVA. The samples to be excluded were chosen with the assistance of a random numbers table (Rohlf and Sokal, 1981). Details of statistical tests and results may be found in figure legends. Higher-order interactions themselves will only be discussed if significant. In cases where no significant higher-order interactions have been identified through statistical analyses, the results of main effects will be shown individually to simplify presentation and facilitate interpretation of the results.

### 3. Results

#### 3.1. Field observations of experimental colonies

All *S. ehrenbergi* colonies survived both the preliminary acclimatization transplant and introduction to the experimental micro-atolls. They also gained biomass and regenerated

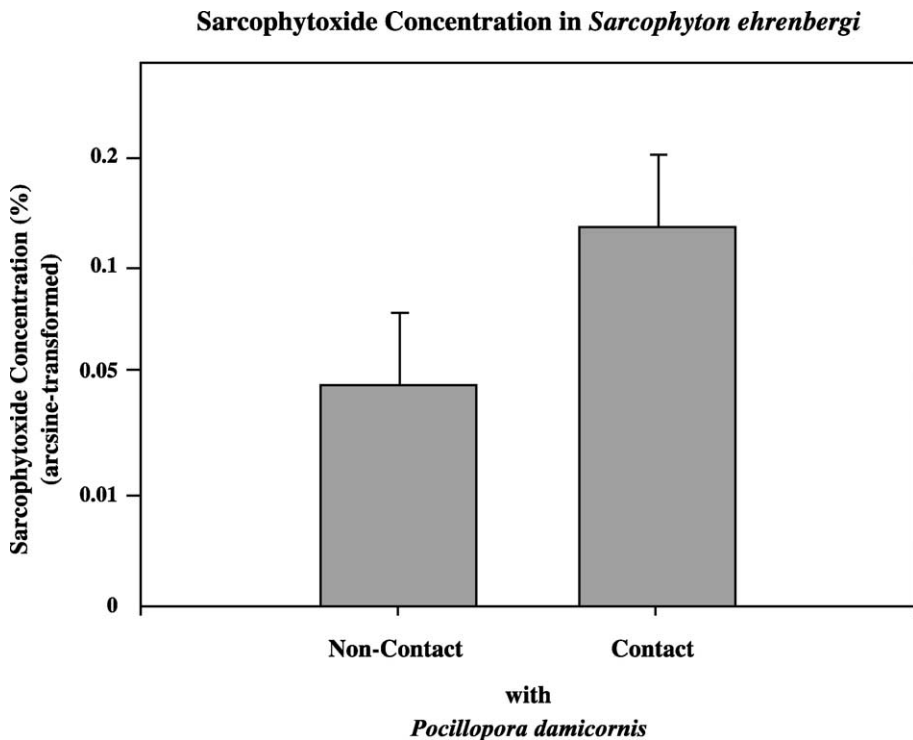


Fig. 1. Concentration of sarcophytoxide, a complementary (secondary) metabolite, in *S. ehrenbergi*. Levels shown under conditions where the soft coral was placed in contact with the scleractinian coral *P. damicornis* vs. non-contact (control) conditions. Concentrations shown in percent. Mean with 95% confidence limits shown.  $n_i = 12$ . Data transformed by arcsine for purposes of normalization. Significant difference found between contact and non-contact concentrations ( $p < 0.001$ , four-way ANOVA). No significant higher-order interactions were found between contact, nutrient-addition, reef and time; therefore, data have been summed over these main effects and presented accordingly.

well between sampling times. Several colonies were observed to secrete mucus sheets on their polypary surfaces, a natural phenomenon reported elsewhere (Coffroth, 1985; Coll et al., 1987). Asexual reproduction by fragmentation, budding, and stolon formation was also observed during the course of the study. Two-thirds of the *P. damicornis* colonies used in the contact vs. non-contact experiment died during the course of the experiment (March 1995,  $n = 36$ ; February 1996,  $n = 11$ ).

### 3.2. Manipulative experiments

Sarcophytoxide levels in *S. ehrenbergi* varied greatly in concentration between colonies in this experiment (0.01–0.61% dry weight). In considering the effect of competition for space on the concentration of this metabolite, there was a highly significant effect of contact between the soft coral and scleractinian coral on sarcophytoxide concentration (Fig. 1). On the average, sarcophytoxide concentrations were three times higher in

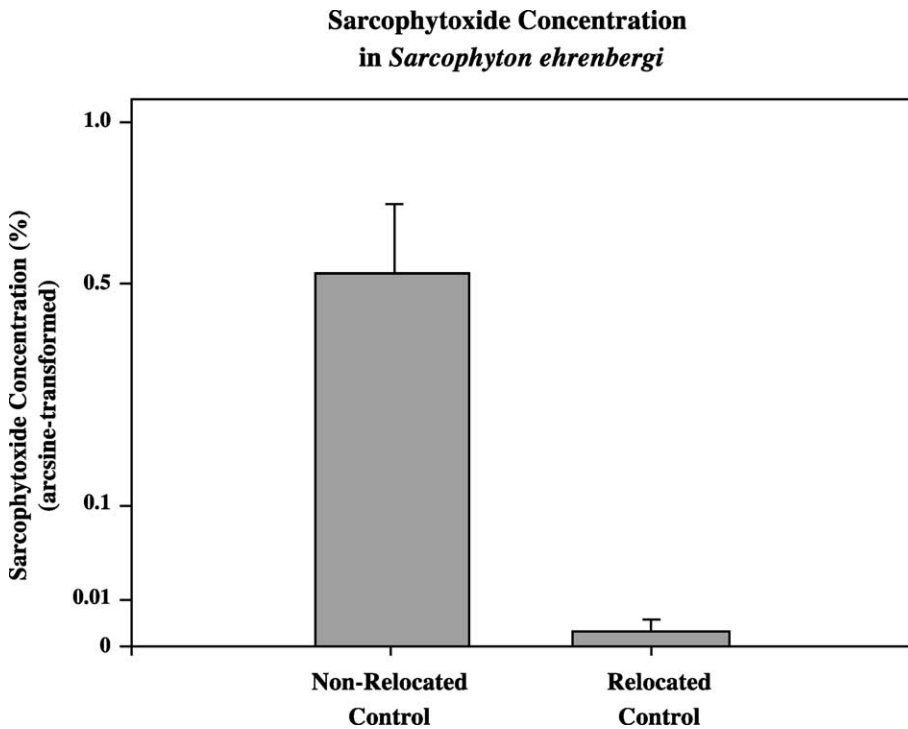


Fig. 2. Concentration of sarcophytoxide in *S. ehrenbergi*. Levels shown under conditions where the soft coral has been relocated from a source area at the lagoon entrance at One Tree Island to 12 micro-atolls. Concentrations shown in percent. Mean with 95% confidence limits shown. Data transformed by arcsine for purposes of normalization. Significant difference found between relocation and non-relocation concentrations ( $p < 0.01$ , two-way ANOVA). No significant effect of time was found ( $p > 0.05$ ) nor was there any significant higher-order interaction; thus, significant main effect data are shown here, summed over time.

colonies under contact conditions than in the controls. Some of the *P. damicornis* colonies died during the course of the experiment, leaving only the skeleton behind, still in contact with the soft coral. In those cases, no further increase in sarcophytoxide was observed in the interacting *S. ehrenbergi* colonies.

The most dramatic shifts in sarcophytoxide concentration in this experiment occurred in the control colonies, which were transplanted to micro-atolls. Sarcophytoxide concentrations decreased significantly and dramatically in transplanted *S. ehrenbergi* colonies, compared to the non-relocated control colonies ( $p < 0.01$ , two-way ANOVA; Fig. 2). Colonies in their natural environment (non-relocated controls) produced higher levels of sarcophytoxide (0.42–0.61% dry weight) than relocated colonies, whether growing in

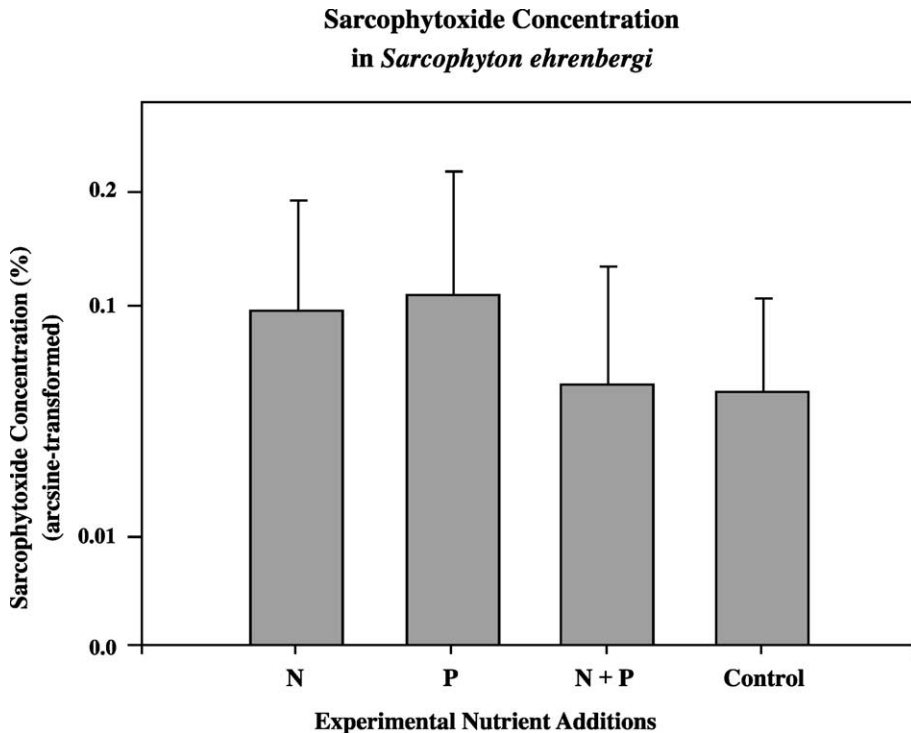


Fig. 3. Concentration of sarcophytoxide in *S. ehrenbergi*. Levels shown under conditions where soft corals have been transplanted to micro-atolls subjected to additions of nitrogen (as ammonium chloride,  $\text{NH}_4\text{Cl}$ ), phosphorus (as potassium di-hydrogen phosphate,  $\text{KH}_2\text{PO}_4$ ), and N and P together, respectively. Control micro-atolls without nutrient additions were also used. Concentrations shown in percent. Mean with 95% confidence limits shown. Data transformed by arcsine for purposes of normalization. Significant overall difference in sarcophytoxide concentrations between nutrient-addition treatments ( $p < 0.05$ , four-way ANOVA). This significance was not strong; however, a posteriori multiple comparison of means indicated no significant differences between individual means using the T, T', Tukey–Kramer, GT2, Games and Howell, Welsch Step-Up and SS-STP methods ( $p > 0.05$  in all cases). No significant effects of reefs or time ( $p > 0.05$ ). No significant higher-order interactions were found; therefore, data have been summed and presented accordingly.

isolation (0.01–0.21% dry weight) or interacting with *P. damicornis* (0.08–0.28% dry weight; Fig. 2).

There was no significant change in sarcophytoxide with respect to time when considering the relocation vs. non-relocation controls ( $p>0.05$ , two-way ANOVA). There was also no significant overall change in sarcophytoxide production when averaged over all treatments or through time, over the period of the study ( $p>0.05$ , four-way ANOVA).

There was a significant difference in mean sarcophytoxide concentrations within the soft corals overall between the various nutrient addition treatments and control ( $p<0.05$ , four-way ANOVA; Fig. 3). The response, however, was not necessarily a strong one. More conservative a posteriori statistical analyses were unable to reveal significant differences between means when tested individually against each other ( $p>0.05$ , T-, T', Tukey–Kramer, GT2, Games and Howell methods, etc.; see legend Fig. 3). That is, the main effect was significant, but weak. It would appear that the

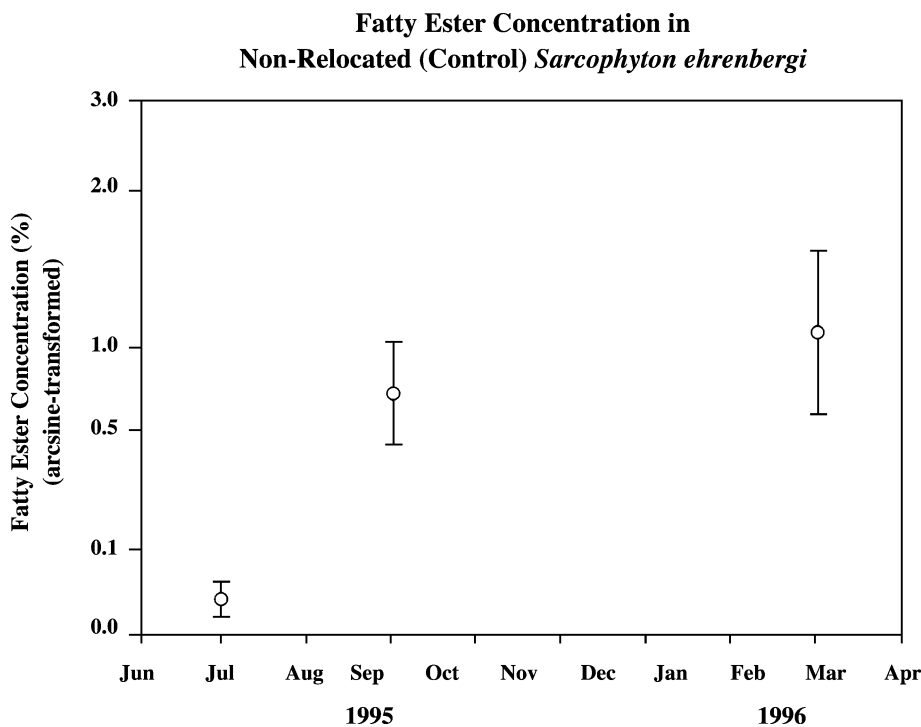


Fig. 4. Concentration of fatty esters in *S. ehrenbergi* through time, in control (non-relocated) colonies. Concentrations shown in percent. Mean with 95% confidence limits shown. Data transformed by arcsine for purposes of normalization. Significant difference in fatty ester concentrations through time ( $p<0.001$ , two-way ANOVA). Significant increase in fatty ester concentrations through time as well ( $r=0.5983$ ,  $p<0.01$ , Pearson's product-moment correlation;  $p<0.05$ , linear regression analysis,  $Y=0.262X+4.704$ ; but see Fig. 5 and discussion in text).

nitrogen-addition and the phosphorus-addition treatments were both driving the significant increase in sarcophytoxide production within the soft corals. Addition of a combination of nitrogen and phosphorus together produced an average sarcophytoxide concentration closer to that of the control, but with a high enough variance to not vary greatly from either the other nutrient-addition treatments or the control ( $p > 0.05$ , four-way ANOVA).

With respect to fatty esters, the production of this suite of metabolites generally did not change through time in the main treatments. A temporal effect was revealed, however, in some of the controls (Figs. 4 and 5). Specifically, fatty ester production increased significantly through time in the control (non-relocated) colonies (Fig. 4;  $p < 0.001$ , two-way ANOVA;  $p < 0.01$ , Pearson's product-moment correlation,  $r = 0.5983$ ;  $p < 0.05$ , linear regression analysis). No such significant change occurred through time in the relocated colonies (Fig. 5;  $p > 0.05$ , Pearson's product-moment correlation;  $p > 0.05$ , linear regression analysis). Fatty ester concentration was originally low in the non-relocation control colonies ( $< 0.1\%$ ) but increased significantly, with the major jump in concentration occurring between July and September 1995 (winter; Fig. 4). This is the only response in

#### Fatty Ester Concentration in Relocated *Sarcophyton ehrenbergi*

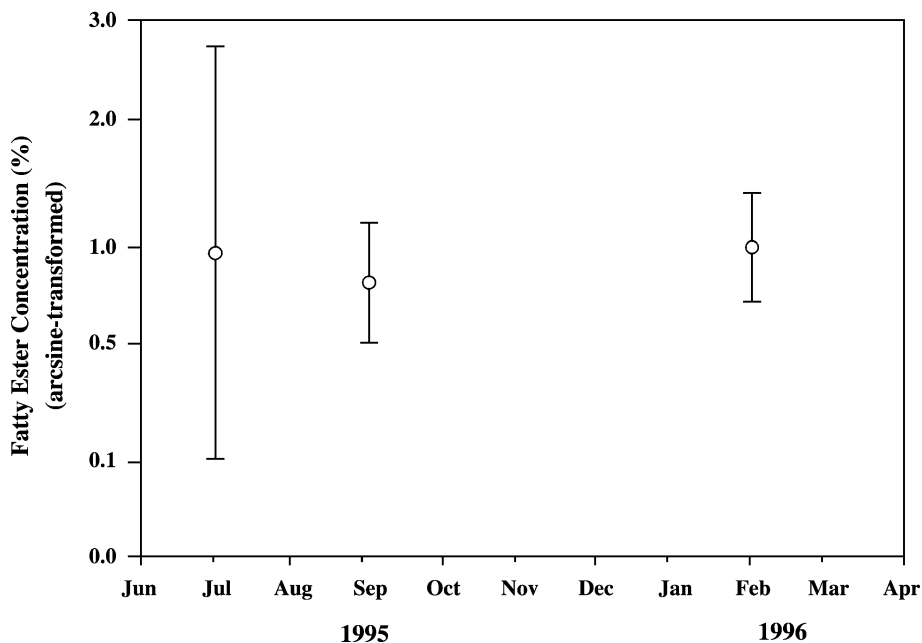


Fig. 5. Concentration of fatty esters in *S. ehrenbergi* through time, in relocated colonies, control group. Concentrations shown in percent. Mean with 95% confidence limits shown. Data transformed by arcsine for purposes of normalization. No significant difference in fatty ester concentrations through time ( $p > 0.05$ , four-way ANOVA). Significant two-way interaction with relocation ( $p < 0.05$ ). (Also see Fig. 4).

the study in which time was determined to significantly influence the concentration of compounds being measured.

As a main effect, there was no significant difference between fatty ester concentrations in relocated vs. non-relocated (control) colonies ( $p > 0.05$ , two-way ANOVA). For the reasons cited above, there was a significant higher-order interaction between relocation and time ( $p < 0.05$ ).

Soft coral colonies placed in contact with *P. damicornis* exhibited significantly lower concentrations of fatty esters than the control soft coral colonies not in contact with the scleractinian coral (Fig. 6).

Fatty ester concentration in *S. ehrenbergi* also varied significantly with the addition of nutrients to the local environment (Fig. 7). Most striking was the increase in fatty ester concentration with the addition of phosphorus alone. The addition of nitrogen alone

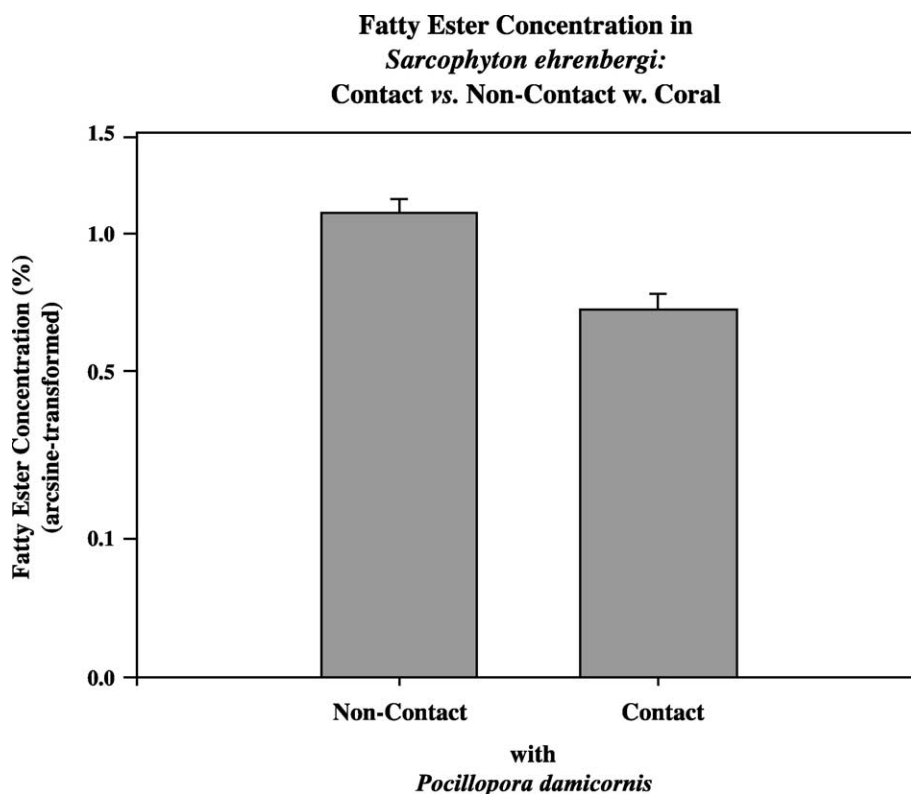


Fig. 6. Concentration of fatty esters in *S. ehrenbergi*. Levels shown under conditions where the soft coral was placed in contact with the scleractinian coral *P. damicornis* vs. control/non-contact conditions. Concentrations shown in percent. Mean with 95% confidence limits shown. Data transformed by arcsine for purposes of normalization. Significant difference between contact and non-contact concentrations ( $p < 0.001$ , four-way ANOVA). No significant higher-order interactions were found; therefore, data have been summed over other main effects and presented accordingly.

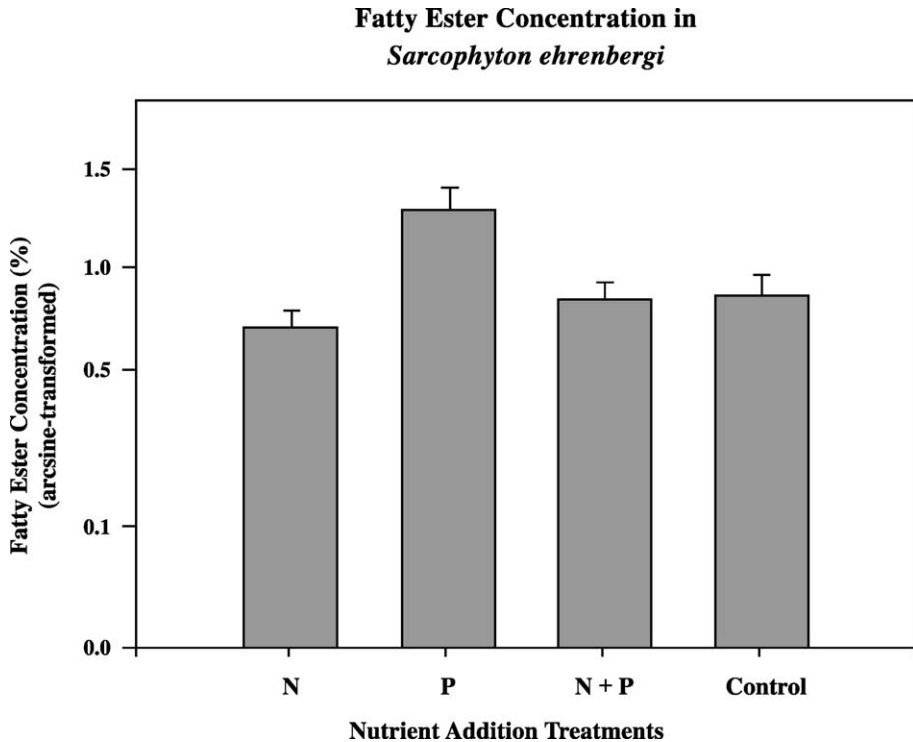


Fig. 7. Concentration of fatty esters in *S. ehrenbergi*. Levels shown under conditions where soft corals have been transplanted to micro-atolls subjected to additions of nitrogen (as ammonium chloride,  $\text{NH}_4\text{Cl}$ ), phosphorus (as potassium di-hydrogen phosphate,  $\text{KH}_2\text{PO}_4$ ), and N and P together, respectively. Control micro-atolls without nutrient additions were also used. Concentrations shown in percent. Mean with 95% confidence limits shown. Data transformed by arcsine for purposes of normalization. Significant difference in sarcophytolide concentrations between nutrient-addition treatments ( $p < 0.001$ , four-way ANOVA). No significant higher-order interactions were found; therefore, data have been summed over the other main effects and presented accordingly.

actually resulted in a significant decrease in the production of fatty esters and the addition of both nitrogen and phosphorus resulted in no net change in fatty esters.

#### 4. Discussion and conclusions

This report provides experimental evidence for the induction of terpenoid biosynthesis caused by changes in the nature of interspecific competition. It also provides evidence for either the inhibition of wax ester biosynthesis or the higher metabolism of wax esters under these same conditions. A previous study had suggested that the chemical composition of the soft coral *Sinularia flexibilis* was a function of the competitive nature of the soft coral's environment (Maida et al., 1993). This study experimentally confirms a critical relationship between inter-specific competition for space and terpene biosynthesis; that is, a phytoalexin is being produced in response to direct contact with a competitor for space.

(Phytoalexin is a botanical term meaning a toxic compound produced generally by higher plants in response to attack by pathogens and to other stresses, sometimes referred to as a plant antibiotic; these compounds are considered to be non-specific and have a general fungicidal and/or bacteriocidal action. See Harborne [1999] and Grayer and Kokubun [2001] for recent reviews).

*S. ehrenbergi* clearly thrives in the micro-atolls, an environment where they are not readily found. The good health and condition of the soft coral colonies transplanted into this habitat was indicated by the extent of their asexual reproduction. There, the colonies fragmented, grew stolons to expand into surrounding space, and budded off smaller colonies which were established around the experimental grids (see Dinesen, 1980; Acosta, 1999; Acosta et al., 2001, in press).

Competition is known to be a major selective force in structuring benthic marine communities (e.g., Connell, 1983). In particular, competition for space can be strong enough to cause total local exclusion of species (Hughes et al., 1987; Alino et al., 1992). At initiation of exposure to experimentally increased nutrient concentrations, some of the experimental *S. ehrenbergi* colonies were in contact with *P. damicornis*. By the end of the experiment, 69% of the *P. damicornis* colonies had died as a result of contact with, or proximity to, *S. ehrenbergi*. *P. damicornis* occurred naturally on the micro-atolls. It is unlikely that their mortality was due to the local micro-atoll environment; rather, mortality was most likely due to contact with *S. ehrenbergi*. *Pocillopora* was not significantly affected by the nutrient additions to these micro-atolls, as determined via parallel observations made within the same experimental system at the same time (Hoegh-Guldberg, in press; Koop et al., 2001). Indeed, during the ENCORE experiment, *P. damicornis* exhibited an annual mortality frequency of only 17–22% when relocated into the micro-atolls (Hoegh-Guldberg, in press). This is in contrast to the 69% mortality of *P. damicornis* documented here, when these colonies were interacting with *S. ehrenbergi*. The intensity of inter-specific competition for space and its effects on sarcophytoxide production in particular was found to be virtually independent of the nutrient regime.

The high mortality rates in *P. damicornis* were associated with increased levels of sarcophytoxide in *S. ehrenbergi* (Fleury et al., 2000). As the levels of sarcophytoxide increased in the soft coral, mortality in *P. damicornis* increased. Once the *P. damicornis* colonies were dead, there was no further increase in the concentration of sarcophytoxide in *S. ehrenbergi* colonies. Although sarcophytoxide is a minor chemical component of the tissue of *S. ehrenbergi* at One Tree Island, it is highly bioactive (Kobayashi et al., 1983; Kung and Ciereszko, 1977; Pass et al., 1989). It is a known fish toxin (Tursch et al., 1978) and has been implicated as an allelopathic agent in inter-specific interactions with scleractinian corals (Webb and Coll, 1983).

Naturally occurring *Sarcophyton* colonies (non-relocated colonies) produced significantly higher levels of sarcophytoxide than relocated colonies interacting with *P. damicornis*. The relocated control colonies (which were not placed in a competitive interaction with *P. damicornis*) experienced a dramatic and highly significant decrease in sarcophytoxide production after transplantation. In the study area, *S. ehrenbergi* colonies compete for space with a wider range of organisms in their source locale (lagoon entrance; Fleury, 1999). On the experimental micro-atolls to which they were relocated,

however, they were set up to compete with only one experimental species—*P. damicornis*. In addition, the non-interacting relocated colonies of *S. ehrenbergi* were isolated from other inter-specific interactions on their segregated grids. This would have resulted in a reduced need to produce allelochemicals for defense. Although a number of factors may influence metabolite concentration, the only ones we manipulated here were competition for space and nutrient concentration, and responses were received from both of these.

Transplanting colonies in the field can represent a significant disturbance to corals (Willis, 1987; Timson, 1987; Seddon, 1989). We do not believe, however, that this factor caused any changes in metabolite levels. A substantial amount of time was permitted to pass between collection of the experimental colonies, their relocation to the experimental sites, and the measurement of complementary metabolites. Sampling for, and analysis of, the compounds began about 1 year after relocation, allowing for any necessary healing of tissue after collection and for acclimation to the new habitat. Leone et al. (1995) have shown that relocation does have an initial elevating effect on the concentration of complementary (secondary) compounds. This stress response usually lasts about 2 months, and then the elevated concentrations of metabolites then return to their pre-relocation levels. Photographic records and field observations over this period showed that only a few *P. damicornis* colonies did not survive the set-up procedure; these were all replaced as needed. All *S. ehrenbergi* colonies survived the relocation, gained biomass and reproduced asexually throughout the study. This suggests that the relocation did not represent a source of stress for the soft corals.

The addition of nitrogen and phosphorus, respectively, significantly enhanced the production of sarcophytoxide in the tissues of *S. ehrenbergi*, under conditions of both contact and non-contact with *P. damicornis*. The overall effect was significant, but weak. The effect of the individual nutrient additions was stronger than the addition of both nutrients together. This is a preliminary indicator that not only is competition for space perceived as a stress for the soft coral, but so is nutrient enrichment in the local environment—for both N and P. Thus, the organisms may be disadvantaged in the longer-term under conditions of nutrient enrichment by having a demand placed upon them for higher levels of complementary metabolites for long periods of time. Interestingly enough, exposure to a combination of N and P together produced no marked response in sarcophytoxide production, except for an increase in variance from the controls. It is possible that the two nutrients somehow cancel each other's individual effects to produce an overall null effect. This question remains open and requires further investigation.

The fact that fatty ester concentrations in control soft corals increased towards the end of winter implies that biosynthesis of these energy storage compounds may naturally increase at this time. The changes could not have been influenced by bleaching, because no bleaching was observed during the course of the experiment; in addition, seawater temperatures were low at the time when the increase in fatty ester production was noted—during the winter. Whether the increase in concentration was because it is part of a natural seasonal cycle or is the response to some stimulus in the local environment is not known. What is known, however, is that production of wax esters in the relocated soft corals was high directly after initiation of the experiment and remained so throughout the experiment.

The variance in wax ester production was also high in relocated colonies. The implication here is that these energy storage compounds were synthesized in response to the relocation and that production remained high, equaling that of the non-relocated colonies. The experiment was able to differentiate, however, between natural increases in the production of fatty esters and concomitant, disproportionate decreases due to contact with *P. damicornis*. It was also able to differentiate between natural changes in concentration and changes due to the addition of nutrients to the local environment.

Placing *S. ehrenbergi* colonies into direct contact with colonies of the scleractinian coral *P. damicornis* resulted in a decreased concentration of fatty esters in the soft coral. Such decreases in these energy storage compounds indicate the stress caused by contact between the soft corals and scleractinian corals. Changes in the biosynthetic pathways of the relocated *S. ehrenbergi* colonies may well have been taking place here. The interaction between the two colonies appears to have caused a stress on the soft coral and an increased demand for the diterpene sarcophytoxide, used in defense of space. This was indicated by its increasing concentrations of the compound. This, in turn, increased the cost of terpene production and probably increased the demand on energy storage supplies in the form of waxy esters, causing a decrease in their concentrations. This agrees with the findings of Fleury et al. (2000). The soft coral's ability to manufacture energy reserves at the pre-competition level for future needs may also have been affected, but this remains to be confirmed.

The addition of phosphorus to some of the experimental micro-atolls clearly caused an increase in the concentration of wax esters in the associated soft coral tissues. It is possible that this response may be attributed to the role of phosphorus in the deposition of phospholipids in biomembranes, and the biosynthesis and storage of acylated glycerol derivatives and wax esters which are dependent on phosphate-derived intermediates (Garrett and Grisham, 1999, pp. 819–823). Increasing bioavailability may permit increased energy storage, mainly in the non-interacting colonies. An increase in phosphorus in the environment is generally construed as a source of stress, and it is also possible that this change in environmental conditions is perceived by the organism as an indicator for the need to manufacture additional energy storage compounds to assist in dealing with that stress, perhaps because of increased metabolism.

The study shows that *S. ehrenbergi* colonies are able to alter their metabolism under stress, increasing the production of sarcophytoxide, an allelopathic agent, at the expense of fatty esters, energy storage compounds. This process contributes to the maintenance of the high populations of *S. ehrenbergi* colonies in this ecosystem. These interactions help to explain the dominance of *S. ehrenbergi* populations in certain local habitats in this region.

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